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SCIENTIFIC RESEARCH ON PAPAVER BRACTEATUM [1]

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ANALYSIS OF PAPAVER ALKALOIDS AND STABILITY OF THEBAINE [2]

by: F.J.E.M. Küppers, R.J.J.Ch. Lousberg,
C.A.L. Bercht and C.A. Salemink,

Organic Chemistry Laboratory,
State University,
Utrecht, The Netherlands.

1/ General note: The views expressed by collaborating scientists in their contributions to the programme are not necessarily shared by the Secretariat.

2/ In the report of the Second Working Group on Papaver bracteatum (document ST/SOA/SER.J/2), it was recommended that methods for the determination of thebaine in Papaver bracteatum should be compared and evaluated; to achieve this, it was decided that participants in the Working Group would forward their methods for publication in this series so that these could be tried out in the various laboratories. It was also recommended that a study be made of the stability of thebaine.

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Within the scope of our work on opium alkaloids, we became particularly interested in the botanical and chemical aspects of Papaver bracteatum. Detailed motives for this special interest are described in the first two reports in the United Nations series on scientific research on Papaver bracteatum[1a,b] and in the interesting article by Fairbairn and Hakim[2] on P. bracteatum Lindl., as a new source of opiates.

From the above reports, it was concluded t at immediate attention should be given inter alia to chemical aspects such as the extraction and analysis of P. bracteatum and the stability of thebaine, one of the major constituents of several races of P. bracteatum. The present study concerns these last aspects.

Chromatographic analyses of opiates

Gas chromatography. Several methods have been investigated for the qualitative and quantitative analysis of the alkaloids, such as those described by Brochmann-Hanssen[3], Nieminen[4], Cheng[5] and Jones[6]. It was considered important for good quantitative analysis that the morphine and thebaine should be well separated. The following compounds were used as internal standards: histapyrrodine hydrochloride, estradiol valerate and cholesterol acetate. The solid support was either 60-80 mesh or 100-120 mesh.

The best results with regard to reproducibility, separation and tailing were obtained with 2 per cent OV-17 on Chromosorb-G AW-DMCS 100-120 mesh at temperatures between 260-300°. It was found that the solid support of 60-80 mesh caused considerable tailing. The instrumental conditions were:

gas chromatograph	: Becker 409
detection	: F.I. and digital integrator
column	: 6 ft. glass, inner diameter 3 mm, 2 per cent OV-17 chromosorb-G AW-DMCS, 100-120
injector temperature	: 290°
detector temperature	: 310°
oven temperature	: 25 min. isotherm at 270°, next 5°/min. until 290° and finally isotherm at 290°
carrier gas	: nitrogen, 2.5 atm. inlet pressure, flow rate approx. 45 ml/min.

A sample of P. bracteatum (UNB 6) was extracted by

(a) the method of Nieminen[4] (treatment with water at pH 10.0 and extraction with chloroform. The combined chloroform layers were dried over $MgSO_4$, evaporated to dryness and re-dissolved in methanol)
(b) the method of Fairbairn[2] (extraction with ammoniacal methanol, evaporation to dryness and re-dissolving in methanol).

Under the above gas chromatographic conditions, the sample analysed as indicated in Table I.

Table I

Sample UNB 6	Retention time in min. (RT)	Relative retention time (RRT)	Relative integration value from integrator counts
M^+ m/e 313;298	8.4	0.45	0.02
M^+ m/e 254;239	12.6	0.67	0.04
Thebaine	18.0	1.00	1.00
M^+ m/e 297	22.0	1.20	0.04

Thebaine was found to be present in a concentration of 0.4 per cent. It seems that the method of extraction is of great importance. The investigations are continuing and further details will be presented in a forthcoming report.

Thin layer chromatography. Different tlc systems were used e.g. the methods of Fairbairn[2] (SiO_2 and benzene-methanol 4:1), the standard system "S 1" of Pfeiffer[7] (SiO_2 and benzene-acetone-methanol 7:2:1) and the method described by Stahl[8] (SiO_2 and toluene-acetone-ethanol-ammonia 25 per cent 40:40:6:2). Detection of the alkaloids was carried out with the iodoplatinate spray reagent[9]. Fertigplatten Kieselgel Merck were used.

Comparison of the above systems clearly showed the first two to be less favourable, because of the unavoidable tailing of the different spots. The system of Stahl gave excellent results and was further used during the following experiments. After one run, the Rf-values for morphine, codeine, thebaine and papaverine were 0.12, 0.20, 0.35 and 0.60, respectively, with the following colour reactions immediately after spraying: gray-violet, violet, blue-violet and red-violet and, after several hours, brown-violet, violet, brown-violet and yellow, respectively.

Analysis of thebaine samples and choice of material. Thebaine*/ and its hydrochloric acid salt**/ were first analysed using the above chromatographic systems. Both products were in white crystalline form.

	GLC RRT.	%	TLC Rf.
Thebaine	0.96	0.2	-
	1.00	99.8	0.35
Thebaine HCl	0.96	0.1	-
	1.00	99.9	0.35

It has been mentioned by Brochmann-Hanssen[10] that both thebaine and its HCl-salt can be used in gas chromatography. Decomposition of the salt in the injector gives thebaine as indicated by the above data. However, we found that the quantitative analysis of the salt is far less reproducible ($\pm$ 30 per cent) than in the case of the free base ($\pm$ 5 per cent). This observation can certainly be ascribed to variations in the degree of decomposition of the salt. For quantitative thebaine analysis, it is advisable to adjust the aqueous thebaine-salt solutions to pH 9 with ammonia and to extract at least twice with chloroform.

The optimal response in the gas chromatographic analysis of thebaine was obtained under the following conditions:

electrometer : range 1, attenuation 16
flame : H_2 pressure 0.75 kg/cm^2
air pressure 2.00 kg/cm^2
carrier gas : N_2 flow rate 45 ml/min.
column temperature : 270°

The absolute response towards 1 µg thebaine was 4.8 cm^2 peak area.

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*/ Obtained from the Nederlandse Fabriek voor Farmaceutische Producten, The Netherlands.

**/ Obtained from the Onderlinge Farmaceutische Groothandel, The Netherlands.

Table II

	Retention time in min. (RT)	Relative retention time (RRT)	Relative integration value from integrator counts
Codeine HCL	11.0	0.61	0.8
Morphine	13.3	0.74	1.0
Thebaine	18.0	1.00	1.3
Papaverine	32.0	1.78	0.9

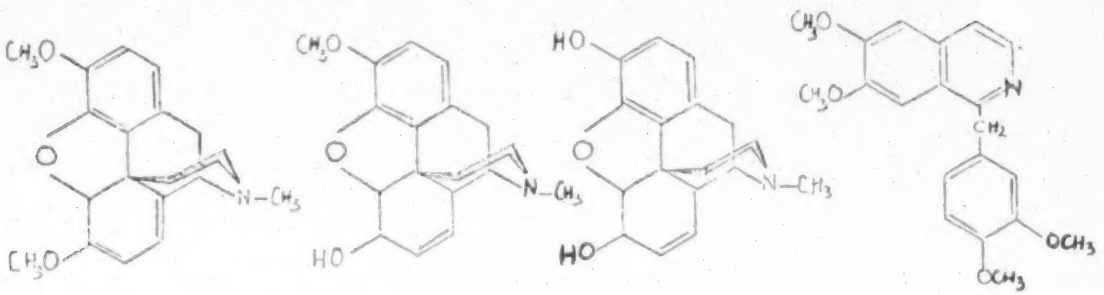
Combined gas chromatography-mass spectrometry of opiates. Analysis of unknown opiates or degradation products in stability experiments can only effectively be carried out by methods which provide both quantitative and qualitative information. Therefore, in addition to the above chromatographic methods, we studied the application of combined gas chromatography-mass spectrometry (GCMS). The experiments were carried out with a JEOL-1100 gas chromatograph equipped with a modified JEOL JMS-07 mass spectrometer (with a double stage jet separator) for the detection.

For orientation, the following substances were first measured: morphine, codeine, heroin, thebaine, narcotine, narceine, papaverine and protopine.

The conditions were as follows:

column : 3 per cent OV-17, Chromosorb-G AW-DMCS
column temperature : 225°
pre-pressure : He, 1.05 atm.
source temperature : 230°
accelerating voltage : 3.0 kV
trap current : 300 µA
electron energy : 70 eV

Characteristic fragment ions and their relative intensities of the four major opiates thebaine (I), codeine (II), morphine (III) and papaverine (IV) are:



I II III IV

I II III IV

I. M/e 311 (100%), 296 (58%), 282 (5%), 280 (4%), 268 (7%), 267 (8%), 265 (4%), 254 (8%), 253 (6%), 250 (3%), 242 (12%), 239 (10%), 211 (9%), 182 (6%), 165 (7%), 58 (14%), 44 (10%), 42 (44%).

II. m/e 299 (65%), 298 (6%), 284 (4%), 282 (6%), 242 (5%), 229 (25%), 214 (15%), 188 (18%), 162 (45%), 124 (32%), 115 (24%), 96 (7%), 94 (12%), 91 (10%), 81 (26%), 59 (38%), 44 (48%), 42 (100%).

III. m/e 285 (100%), 270 (4%), 268 (14%), 256 (5%), 242 (4%), 228 (7%), 215 (34%), 174 (17%), 162 (42%), 124 (30%), 115 (18%), 70 (26%), 44 (48%), 42 (76%).

IV. m/e 339 (32%), 338 (35%), 324 (39%), 315 (6%), 308 (10%), 220 (100%), 205 (16%), 154 (8%), 147 (7%), 91 (8%), 77 (10%).

These products can be well recognized by their molecular weight and they possess molecular ion peaks of moderate to strong intensity.

Stability experiments

Preliminary stability experiments. At first, relatively vigorous conditions were applied. Thebaine-HCl (10 mg) dissolved in 0.5 ml of:

(a) 0.5 N HCl

(b) 1 M tartaric acid

(c) 2% H_2SO_4

was kept for 24 hr at 95-100° in sealed tubes.

Free thebaine (10 mg) suspended in 0.5 ml of:

(d) 0.5 N NaOH

(e) saturated Na_2CO_3 solution

(f) 10% NH_3/H_2O

was treated under similar conditions.

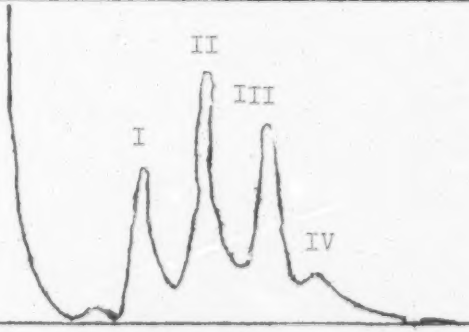
After 24 hours, the acidic reaction mixtures were adjusted to pH 9-10 with ammonia and all reaction mixtures were extracted with chloroform. The chloroform was evaporated and the residue dissolved in methanol. GLC and TLC analyses of the methanolic solutions revealed that thebaine was resistant towards treatments (d), (e) and (f). Treatments (a), (b) and (c) converted thebaine quantitatively into at least four different products.

Stability of thebaine towards 0.01 N HCl. Thebaine-HCl was dissolved in 10 mg amounts in 0.2 ml 0.01 N HCl in sealed tubes (0.5 x 10 cm). Two series of experiments were made:

A. at 90° for (1) 1 hr; (2) 2.5 hr; (3) 5 hr; (4) 8 hr; (5) 12 hr

B. for 12 hr at (1) 30°; (2) 40°; (3) 50°; (4) 60°; (5) 70°; (6) 80°.

Sample A5 was analysed by GCMS and the results are given in the following table.

Total ion current chromatogram	Characteristic mass fragments and their relative intensities
	I. 313 (100%); 298 (15%); 296 (20%); 256 (70%); 255 (65%); 223 (45%) II. 311 (12%); 296 (8%); 280 (45%); 265 (100%); 250 (20%); 235 (30%) III. 293 (100%); 278 (65%); 265 (20%); 250 (40%); 235 (50%) IV. 291 (15%); 276 (10%)*/
RRT 0.76/1.00/1.30/1.61	

*/ These data are difficult to interpret due to the tailing of product III.

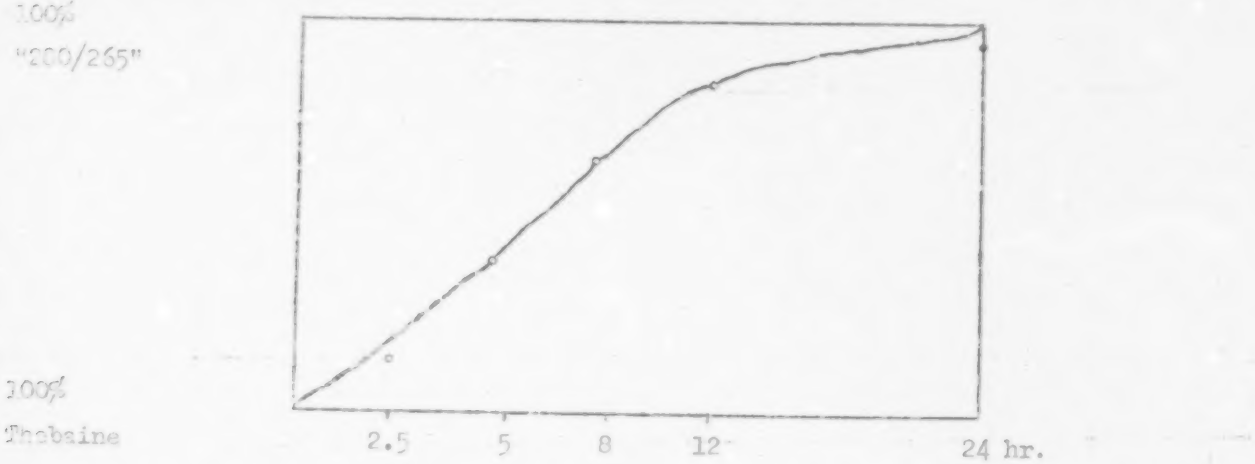
TLC analysis of sample A5 indicated the presence of a weak spot with the Rf-value corresponding to thebaine (0.35) and three further main products with Rf 0.03 (violet with iodoplatinate), 0.72 (light-yellow) and 0.80 (light-yellow). The peak in the ion current chromatogram with RRT 1.00 could, therefore, not correspond to thebaine only.

The same conclusion is provided by comparison of the mass spectrum of peak II in A5 with that of thebaine:

Thebaine	311 (100%); 296 (60%); 280 (4%); 265 (3%); 250 (3%)
Peak 2 in A5	311 (12%); 296 (8%); 280 (45%); 265 (100%); 250 (20%)

After a longer reaction time (24 hr) and at a higher reaction temperature (95°) it was found that the peak with RRT 1.00 consisted only of a product with M^+ m/e 280 and with the base peak at m/e 265. Thus, the product seems to lack the nitrogen atom. Further study is in progress.

The relation between reaction time and the ratio thebaine: compound "280" in peak II at a temperature of 90° in a 0.01 N HCl solution is given in the following figure. The ratio was determined by comparison of the intensities of the base peak of thebaine at m/e 311 with that of the "280" compound at m/e 265.

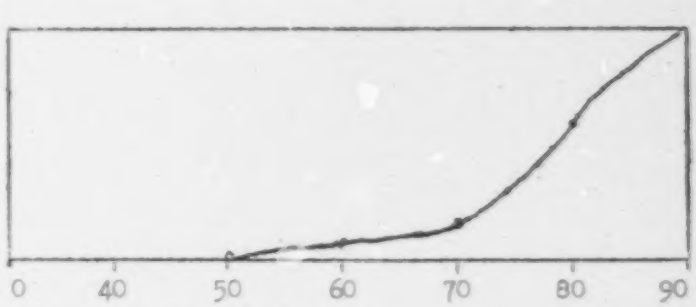


Next to the formation of the "280/265" product, three more substances could be analysed: "313/298", "293/278" and "291/276". The relative formation of these products after different reaction times is given in the next table.

Temperature 90°	1 hr	2.5 hr	5 hr	8 hr	12 hr
"313/298"	-	2%	2.5	3	4
"293/278"	-	4	13	25	40
"291/276"	-	1	1.5	2.5	3
"280/265"	5	12	21	34	44
Thebaine	95	81	62	33.5	9

Stability of thebaine at lower temperatures. The experimental series B with regard to the stability of thebaine towards 0.01 N HCl gave results which can be summarized in the following graph:

% of decomposition products relative to starting material after 12 hr reaction time



The above graph clearly illustrates that, during at least 12 hr, thebaine is stable in 0.01 N HCl at temperatures below 50°.

Stability of thebaine towards several acids used in opiate-processing. The stability of thebaine was investigated towards 0.01 N HCl, 0.1 M tartaric acid, 0.1 M citric acid and 0.5 M acetic acid at 80 and 90° after 12 hr reaction time and addition of a solution of 0.1 M $NaHSO_3$.

Reaction time 12 hr						+ 0.1 M $NaHSO_3$				
	Relative conc. of products					Relative conc. of products				
	I	280	thebaine	III	IV	I	280	thebaine	III	IV
0.01 N HCl 80°	3	36	37	22	2					
0.01 N HCl 90°	4	44	9	40	3	7	28	59	4	2
0.1 M tartaric acid 80°	2	41	48	8	1					
0.1 M tartaric acid 90°	6	49	10	33	2	6	-	94	-	-
0.1 M citric acid 80°	4	1	95	-	-					
0.1 M citric acid 90°	10	42	28	16	4	4	-	96	-	-
0.5 M acetic acid 80°	2	-	98	-	-					
0.5 M acetic acid 90°	4	2	94	-	-	2	-	98	-	-

The results given in the above table clearly indicate the stabilizing effect of the addition of $NaHSO_3$ to the reaction mixture. This is particularly so in the case of the treatment of thebaine with tartaric acid and with citric acid.

Stability of thebaine and its HCl-salt towards oxygen and light. The possible influence of oxygen and light on methanolic solutions of thebaine and its HCl-salt was studied.

During 12 hr, oxygen was bubbled through a solution of thebaine (15 mg) in methanol at a flow rate of 10 ml/min. One solution was kept at room temperature in the dark, a second solution was exposed to light of two TL-tubes at a distance of 40 cm. Neither of the solutions showed measurable decomposition of the thebaine.

The same experiments were carried out with an aqueous solution of thebaine HCl-salt. In the dark, no decomposition was observed. However, under the influence of light, at least 10 per cent of the thebaine salt was converted into the product with typical mass fragments "313/298".

Further experiments:

1. Oxygen (10 ml/min) was bubbled through a solution of 15 mg thebaine HCl in 0.5 ml undistilled water (pH 6) at 80° for 12 hr. Light was provided by two 65 W TL-tubes at a distance of 40 cm.
2. The same experiment was carried out at 25°, using a 0.001 N HCl solution.
3. Similar as for 2, using a 0.01 N NaOH solution.

Results:

Exp.	RRT	%	MS data
1.	0.52	2	-
	0.76	14	"313/298"
	1.00	82	thebaine
	1.30	2	"293/278"
2.	0.76	3	"313/298"
	1.00	97	thebaine
3.	1.00	99	thebaine

Conclusions

GCMS provides valuable information in the analysis of opiates. Thus it was found that thebaine-HCl could be converted at 90° into a major product possessing identical retention time as thebaine itself. However, from its mass spectrum, it was clearly demonstrated that this product was different from thebaine.

Experiments with regard to the stability of thebaine indicated that:

(a) the temperature to which thebaine is exposed is of great importance;

(b) the pH of the preparation influences the degree of decomposition;

(c) working temperatures below 50° cause only slight decomposition;

(d) addition of $NaHSO_3$ during working processes with thebaine provides considerable stabilization;

(e) oxygen and light affect solutions of thebaine hydrochloride.

References

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(b) United Nations document ST/SOA/SER.J/2 (1973)

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